Aflatoxin Inactivation in Corn by Ammonia Gas: A Field Trial

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FLATOXIN, a potent toxin found in moldy corn, Aacts as a carcinogen, teratogen, and mutagen in laboratory animals. Aflatoxin B₁ content of naturally contiminated corn was reduced from 750 to 7 µg/kg (or ppb) by ammoniation. A 29-t (metric tonne) stationary bed of corn was treated during warm weather with a recycling ammonia-air mixture in a sealed, outdoor metal grain storage-drying bin. The corn was moisture conditioned from 12 to 17.5 percent (w.b.) and 1.5 percent (d.b.) ammonia was added, after which the corn was held 13 days at 32 to 43 °C and then dried to less than 11 percent moisture. The decontaminated corn, an ammoniated good corn, and their unammoniated counterparts are being evaluated in feeding tests with swine and laying hens; these tests are required to obtain FDA approval of such corn. Results from these tests are satisfactory and will be reported elsewhere.

INTRODUCTION

Aflatoxin is recognized as a potent mycotoxin that can occur as a contaminant in various agricultural commodities. Its presence causes a serious problem to the animal production industry. The effects can range from insidious loss of feed efficiency to death of the animals. Several excellent reviews provide further information on the subject (Goldblatt, 1969; Lillehoj

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et al., 1970; Detroy et al., 1971; Butler, 1974; Ciegler, 1975; Christensen et al., 1977).

Ammonia (NH₃) effectively inactivates much or all of the aflatoxin in peanut and cottonseed meals (Gardner et al., 1971; Prevot, 1974) and in corn (Brekke et al., 1977). Chemical assays were used in the above studies to determine the extent of detoxification; subsequent short-term biological tests confirmed a marked reduction in acute toxicity of the aflatoxin (Dollear et al., 1968; Mann et al., 1971; McKinney et al., 1973; Hamilton et al., 1973 and 1974; Brekke et al., 1977a) as has one 12-month test with trout (Brekke et al., 1977b).

Present indications are that use of the NH₃-detoxified corn will be limited to animal feed. The Food and Drug Administration (FDA) currently limits total aflatoxin (B₁, B₂, G₁, and G₂) content of the corn to 20 μ g/kg (i.e., 20 ppb) (Rodericks, 1975). Within the United States, for FDA approval of NH₃-detoxified corn as an animal feed and of the process for preparing such corn, an extensive series of tests is required to demonstrate safety of the NH₃-detoxified corn to animals and of the edible animal tissues and products to humans.

We prepared two lots of ammoniated corn for such feeding tests with swine and laying hens. One lot was from heavily contaminated corn and one from uncontaminated corn. Preparation of two additional lots required only cleaning and blending; these lots were the corn naturally contaminated with aflatoxin (lot AC) and the good corn (lot GC) having no detectable levels of aflatoxin, ochratoxin, and zearalenone. This paper describes the procedure and results obtained in preparation of the ammoniated counterparts (lots ACA and GCA). Conditions used in the field trials were based on data obtained in laboratory experiments (Brekke et al., 1978).

MATERIALS AND METHODS

Corr

Lot AC (Aflatoxin-Contaminated Corn). A blended lot of yellow dent corn, U.S. sample grade, naturally contaminated with 900 μ g/kg total aflatoxin (750 of B₁, 90 of B₂, 40 of G₁, and no G₂ detected) was used. Relative standard deviation (RSD) of B₁ content was 16 percent. (Note: See section on Safety Considerations for information on safety procedures used and some health problems that might be encountered in handling the contaminated corn.)

Lot GC (Good Corn). A yellow dent corn, U.S. grade No. 4, with no detectable levels of aflatoxin, ochratoxin, and zearalenone was used.

Ammonia

The liquid anhydrous ammonia was agricultural grade, minimum purity 99.5 percent.

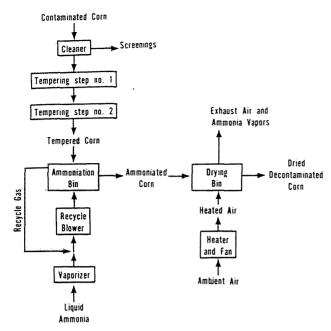


FIG. 1 Flowsheet for inactivation of aflatoxin in corn by ammonia gas at atmospheric pressure.

EXPERIMENTAL PROCEDURES

Preparation of Grain Bins

For blending the corn, we used several 5.5-m diameter galvanized metal grain storage bins, 78 to 102 m³ capacity. The large bin was fitted with a conventional elevated, slotted metal grain-drying floor; a 15-cm diameter under-the-floor grain-unloading auger; and a single, vertical screw bin-stirring device. Some but insufficient blending and some breakage of corn occurred during transfer of the corn from one bin to another. Final blending of the corn (before ammoniation, and after drying the detoxified corn) was completed in the 102 m³ bin equipped with the stirring device.

Two bins similar to the large bin, but of 78 m³ capacity and without the stirrer, were used for ammoniating and drying the corn. Coarse seams in the ammoniation bin were filled with an all-purpose sealing compound

(Nashu Corp., No. 101, Duct Sealer) and all seams were coated internally with roofing cement (Rutland Fire Clay Co., No. 7). Closed cellular-foam weather stripping served as a gasket for the access door in the roof. For corrosion test purposes, all interior surfaces of the ammoniation and drying bins except roof panels and upper portions of the bin walls were coated with epoxy paint to resist ammonia attack.

A 5.6 kW blower, delivering 0.52 m³ air/s (1100 ft³/min) at 50-cm water column pressure, and 20-cm diameter PVC plastic pipe were used to recycle the ammonia-air mixture through the ammoniation bin (Figs. 1 and 2). The drying bin was fitted with a liquified petroleum gas (LPG)-fired heater, 586 kW (2 X 106 btu/h) capacity and five 40-cm diameter ventilators were installed in the roof.

Instrumentation used in the process is also indicated in Fig. 2.

Corn Cleaning and Blending

Fines and small broken pieces of kernels were removed during one pass of corn lots AC and GC through a rotating, single-action corn cleaner. The cleaned corn contained about 0.5 percent broken corn and foreign material (BCFM, i.e., material that passed through 4.76 mm (12/64-in.) round-hole sieve). Breakage during blending, tempering, and transferring the corn increased the BCFM content to an unmeasured level in the ammoniation bin.

Each lot of cleaned corn was blended as two sublots, which were then cross blended.

Tempering

The corn was tempered (i.e., moisture conditioned) from about 12 percent (w.b.) to about 17.5 percent moisture by a two-step addition of tap water in approximately equal amounts, followed after each addition by a holding period for moisture absorption and redistribution within the kernel. Tempering was desirable because detoxification proceeds more rapidly at the higher moisture content. A grain auger fitted with spray nozzles served as the rewetting apparatus. The two-step procedure minimized any nonuniformity in the

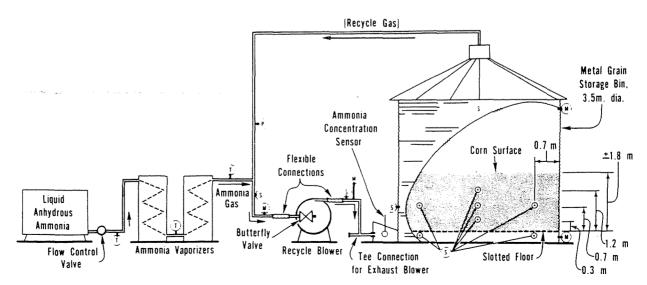


FIG. 2 Schematic diagram of ammoniation bin with recycle piping and blower, ammonia piping and vaporizers, and instrumentation. S = thermistor, T = thermometer, M = manometer tap, and P = pitot tube.

TABLE 1. PROCESS CONDITIONS USED IN FIELD TRIALS

	Run no.	
Item	A	В
Corn lot processed	AC*	GC
Quantity of corn processed:†		
Tonnes (metric)	29.4	25.5
Bushels	1160	1000
Corn M&VM, percent w.b.: ‡		
Initial	12.4	12.0
Tempered	17.4	17.9
Product	9.2	10.1
Ammonia added, percent§	1.48	1.49
Reaction temp, °C:		
Average	36	27
Range	32-39	24-36
Reaction time, days	14.3	13.4
Gas recycling time, h	48	48
Recycle gas flow:		
m ³ /min/m ³ corn	0.8	1.0
_cfm/bu	1.0	1.3
Bed depth, m	1.77	1.55
Aflatoxin B, content, µg/kg:		
Initial corn	750	ND**
Product corn	7	NA††

^{*}AC = aflatoxin-contaminated corn; GC = good corn

tempering and was necessary because the surface of freshly wetted kernels can retain only approximately 3 percent water. Between the two temper steps, the corn was held for a period of 5 to 8 h. It was blended in the blending bin for 5 h or longer after the last water addition before transfer to the ammoniation bin. Usually, 15 to 20 h elapsed between last addition of water and first addition of NH₃.

Ammoniating Corn

Addition of NH₃ to the recycling gas mixture required 5 to 8 h. The ammonia was vaporized in two LPG-fired side-arm heaters, each fitted with 8.2 lineal m of 2.5-cm diameter steel coil. NH₃ vapor concentration in the enriched recycle gas climbed progressively as ammonia was being added and reached a maximum of 75 percent of the lower explosive limit (LEL). (The LEL is 15 volume percent for dry NH₃-air mixtures at 25 °C.) To ensure uniform or near uniform distribution of NH₃ throughout the corn bed, the NH₃-air mixture was recycled for 48 h starting from the time of initial NH₃ addition. Corn remained in the ammoniation bin another 11 days and was then transferred to the drying bin.

Drying Ammoniated Corn

The corn was dried to about 10 percent moisture with air heated to an average temperature of 40 °C (31 to 45 °C range). As reported previously (Brekke et al., 1977a), when average moisture and volatile matter (M&VM) content of the corn was reduced by drying to 11 percent or less, little or no ammonia odor was detectable. After drying, the individual ammoniated corn lots (ACA and GCA) were blended, bagged, and shipped to the groups doing the feeding tests.

Additional information on the process conditions used is given in Table 1.

Safety Considerations for Personnel

Certain safety precautions are necessary in handling both the contaminated corn and the ammoniated corn. Based on our previous experience (Brekke et al., 1977a), we know that exposure to ammonia vapors emitted by the corn can lead to headaches, diarrhea, nausea, temporary weakness, and to a burning sensation when the skin is sweaty. Therefore, wherever the vapors may be present, adequate ventilation and use of respirators is recommended to avoid exposure of workmen to concentrations above the maximum specified by safety authorities. Drawing air down through the bin was the preferred method used when workmen had to work in the bin. Also, as much skin as possible should be covered with clothing to minimize the burning sensation when sweat absorbs ammonia.

The contaminated screenings were treated with lime and buried.

Handlers and users of liquid anhydrous ammonia are or should be familiar with recommended safety practices which need not be reviewed here.

Aflatoxin is a potent toxin that has acted as a carcinogen, a teratogen, and a mutagen when fed to several domestic and experimental animals (Ciegler, 1975). Indirect evidence indicates that the toxin may also produce cancer in humans (Campbell and Stoloff, 1974). Therefore, in handling contaminated grain, proper safety precautions should be followed. Breathing of dust from contaminated grain should be avoided, as should contact of the skin with the dust and the grain. For protection against the aflatoxin-contaminated grain dust and the ammonia vapors, we used the following: dust respirators, respirators fitted with a face shield and NH₃-absorbing cartridges, disposable clothing such as laboratory coats or coveralls made of treated paper, paper caps, and vinyl medical-type gloves.

Clothing such as the above was disposed of either by burial in a commercial land-fill operation or by incineration. Regular work clothing and undergarments were laundered daily on the premises by the work men.

For decontamination, the recommended practice is to wash the exposed skin with household bleach (5-6 percent solution of sodium hypochlorite), preferably full strength, otherwise diluted one-half, and then with soap and water. If the skin is too sensitive to sodium hypochlorite solution, then sodium perborate with a detergent may be used (Goldblatt, 1969).

Grinding of aflatoxin-contaminated samples was conducted in a separate room which has an exhaust fan and a flexible hose to collect dust emission at the hammermill. Ground material was collected in a closed container attached to the mill discharge spout and the container was vented through a dust filter made from two layers of porous cotton fabric.

Equipment such as the sample-grinding mill was housed in a room with adequate ventilation and was decontaminated by washing or wiping necessary surfaces with household bleach, followed by a water rinse.

Sampling and Analyses

For the run on AC corn, we took stream or spot samples (min wt 2.2 kg) as follows for aflatoxin analysis: (a) cleaned, untempered corn—five stream samples; (b) ammoniated corn at bin discharge—six stream samples; (c) corn in ammoniation bin—five spot samples

[†]Corrected to 15.5-percent-moisture basis.

[‡]Moisture and volatile matter: wet basis.

[§] As percent of dry matter in corn.

^{||} From start of ammonia addition to start of drying operation.

^{**}None detected.

^{††}Not analyzed.

TABLE 2. SELECTED ANALYSES OF AMMONIATED CORN

Sample identity	M&VM,* percent w.b.	WE-NH ₃ ,† percent d.b.	NH ₃ -N,‡ percent d.b.	Aflatoxin Β ₁ , μg/kg
	Run No. A			
Bottled samples §	17.8(1)	0.62(3)	0.93(5)	
Vertical bank samples**	16.8(5)	0.46(6)	0.72(2)	3.4(30)
Stream samples††	17.7(4)	0.52(18)	0.80(8)	3.5(36)
		Run	No. B	
Bottled samples	17.9(1)	0.85(2)		_
Vertical bank samples	17.7(3)	0.69(27)	0.87(16)	
Stream samples	18.6(7)	0.71(22)	0.85(11)	

^{*} Moisture and volatile matter.

taken from face of the caked corn; and (d) decontaminated corn—sample from each of five bags in storage after the corn was dried and blended. Each sample was ground (approximately 98 percent passed through a 20-mesh U.S. standard sieve) and blended, and a 1-kg subsample was taken. Single determinations were made by the method recommended for corn (Association of Official Analytical Chemists, 1975; Anon., 1972).

Nine perforated plastic bottles filled with about 200 g of the freshly tempered corn and fastened to individual chains for retrieval were buried at spaced intervals about 30 cm below the corn surface just before the corn was ammoniated. These samples were later analyzed for M&VM and water-extracted ammonia (WE-NH₃) and, after neutralization (Brekke et al., 1977a), for their ammoniacal nitrogen content (NH₃-N).

Spot samples were taken at irregular intervals to determine the amount of moisture added in the tempering step. Four samples (1 kg minimum each) of tempered corn obtained by multiple probes taken vertically in the ammoniation bin at 90 deg intervals about 1.8 m from the bin center were analyzed for their moisture content by both the Cenco and 72-h oven methods. Dry matter content of the corn to be ammoniated was calculated from the average moisture content of the above four samples and a volumetric measurement of quantity of tempered corn in the ammoniation bin.

Moisture content of the untempered corn was determined by a Universal grain moisture tester. Stream samples of tempered corn and the four samples of tempered corn taken by probing the ammoniation bin were coarsely ground in a hand-powered burr mill to pass through a sieve with 2.38-mm openings; the moisture content was based on loss in weight when a 5-g sample was heated 15 min in a Cenco infrared moisture balance. Moisture content of other samples was based on loss in weight when (a) ca. 200 g of whole corn was heated in a 103 °C forced draft oven for 72 h (modification of USDA method 1204, see Anonymous, 1971) or (b) ca.10 g of ground corn was heated for 30 min in a Brabender moisture oven operating at 130 °C. If ammonia was present, the weight loss is reported as M&VM.

Proximate analyses were obtained using approved

procedures for ash, fat, reducing sugars, starch, nonreducing sugars, and sucrose (AACC, 1962; AOCS, 1971; AOAC, 1975). Method for determining fat composition has been described by Black et al. (1967, 1969). The various forms of nitrogen were determined by the procedures reported by Lancaster et al. (1974) and Uhl et al. (1971). Amino acid analyses were made by the Benson and Patterson method (1965). Color was measured with a Hunter Model D-25 color difference meter. Soluble solids were obtained by the Corn Industries Research Foundation method (Anonymous, 1961). Analyses for total bacteria, yeasts, and molds were based on methods outlined by Bothast et al. (1974). Corn samples were graded by the USDA standard procedure (Anonymous, 1970). Sample fragility was measured by use of a Stein breakage tester (2-min agitation of a 100-g sample) with samples tempered to 14 percent moisture by water addition in daily increments of about 2 percent (McGinity, 1970).

RESULTS AND DISCUSSION

Uniformity of Ammonia Application

Good uniformity in ammonia application occurred throughout the bin, based upon chemical analysis of the various corn samples (Table 2). The RSD's of the analyses were remarkably low for the bottle samples and varied between 2 and 27 percent for the vertical bank and stream samples. Clumps or masses of corn exceptionally high in moisture and ammonia content (27 to 49 percent M&VM) created by condensation on the bin roof presumably caused some of the sample-to-sample variation for the latter two types of samples. For example, if one of the vertical bank samples taken in run B is excluded, the RSD's drop to 2, 10, and 4 percent for M&VM, WE-NH₃, and NH₃-N, respectively.

Corn Ammoniation Temperature

Average temperature of lot ACA during the 13-day ammoniation period was 36 °C. While ammonia was being added, average temperature of the bed rose from 29 to 44 °C (Fig. 3). By calculation and assuming no ammonia loss due to leakage, heat of solution of ammonia in the moist corn contributed 12 °C or 80 percent of

[†]Water-extracted ammonia; dry basis.

[±]Ammoniacal nitrogen

[§] Corn in nine perforated bottles buried about 30-cm below surface of corn before ammoniation was started.

^{||} Represents the mean value and relative standard deviation expressed as percent (in parentheses)

^{**}Series of five samples taken at 0.1, 0.4, 0.8, 1.1 and 1.5 m above drying floor from vertical face of caked corn as bin was being emptied.

^{††}Series of six samples (eight for run No. 2) taken at outlet of under-the-floor grain auger as bin was being emptied.

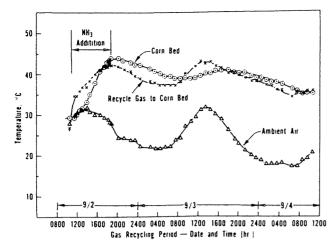


FIG. 3_Temperature profile of corn, recycle gas, and ambient air during ammoniation and gas recycling operations.

this rise. The remaining 3 °C rise is assumed due to the combined effect of energy input from the recycle blower and from solar energy absorbed by the installation. With the recycle blower shut down, the corn bed temperatures averaged 35 to 38 °C during the next 8 days but the vertical temperature gradient increased from 1 to 13 °C. The horizontal gradient never exceeded 3 °C. Operation of the recycle blower for 7 h on day 11 reduced the vertical gradient to 1 °C with the average bed temperature dropping to 32 °C. The decrease occurred in spite of an average 5.7 °C increase in recycle gas temperature across the blower.

The maximum temperature gradient within the corn bed was reasonably small (1.8 °C) while ammonia was being added. The observed small temperature gradient confirms the prediction made by Lancaster et al. (1975) that treatment of a stationary bed of corn with a moving mixture of dilute ammonia and air reduces the non-uniformity in ammonia application resulting from highly localized absorption.

Aflatoxin Inactivation

The decontamination process as used in this field trial was designed to obtain decontaminated corn with an aflatoxin level well under 20 µg/kg. As the data will prove, the process conditions did ensure an effective treatment. The conditions used should not be considered optimum, however. For example, at the reaction temperature attained in treating lot AC, a considerably shorter ammoniation time would certainly appear to have been feasible. This conclusion is based upon the analysis-of two spot samples taken 45 to 60 cm below the surface in the ammoniation bin on days 3 and 5. These samples assayed 3.0 and 4.2 μ g/kg of aflatoxin B₁, respectively. Obviously, the 13-day reaction period used in this run was longer than necessary to bring the total aflatoxin content below 20 µg/kg. Nevertheless, with the swine, laying hen, and rat feeding trials proceeding successfully (Norred, 1977), the more rigorous ammoniation conditions, namely 13-day reaction at an average temperature of 36 °C with 1.5 percent ammonia added to the corn, will define levels which can be used in certain cases where higher aflatoxin levels are encountered or where the aflatoxin is more difficult to inactivate.

The six stream samples taken as lot ACA was removed from the ammoniation bin on day 13 had an average

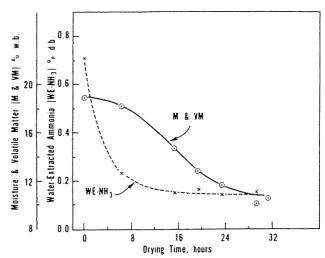


FIG. 4 Batch-in-bin drying of ammoniated corn.

aflatoxin B₁ content of 3.5 μ g/kg (RSD = 36 percent), a reduction of 99.5 percent from the initial level of 750 μ g/kg (lot AC). Average aflatoxin B₁ content of the five samples removed from the vertical bank of caked corn was also low, 3.4 μ g/kg (RSD = 30 percent). Overall, the aflatoxin content of these samples should be considered quite uniform in view of the sampling problem for aflatoxin-contaminated corn and variability of the analytical method, problems that are well recognized by those working in the field.

One spot sample taken as corn was removed from the drying bin also assayed 3.5 μ g/kg aflatoxin B₁, which would indicate that no further reduction in aflatoxin content occurred during the drying step. After 11 months' storage of some of the blended, bagged corn in a barn, five samples of the dried, ammoniated corn assayed 6.8 μ g/kg of aflatoxin B₁ (RSD = 63 percent). However, five samples taken over a 15-month period from batches of lot ACA used to prepare rations fed to swine and laying hens and analyzed by the group supervising the feeding tests had a non-detectable (ND) level of aflatoxin (Norred, 1977). The reason for differences in aflatoxin content of these several sets of samples is not known.

Drying Step

A plot of average moisture content vs time (Fig. 4) gave a typical drying curve for heated air drying of a fixed bed. Total volatiles content of the corn decreased from 18.6 to 10.1 percent in 32 h. Because of the stationary bed and no mixing, a moisture gradient (1.4 to 2.9 percentage points) was observed as might be expected. WE-NH₃ contents of the corn decreased considerably faster than the M&VM. The WE-NH₃ content decreased further from 0.14 to 0.11 percent when corn was removed from the bin and bagged. Some condensate formed on the underside of the roof, but practically all drained off outside the bin.

No scrubber was used for removal of NH₃ vapors from the exhaust gases. Unless one stood directly downstream and close to the bin, the NH₃ odor did not create undue discomfort at any time during the drying step except possibly when the first air exchange took place.

Chemical and Microbial Changes in Ammoniated Corn

Based upon analysis of composite samples prepared from several spot samples taken of the initial and the

TABLE 3. COMPOSITIONAL CHANGES IN CORN*

	Run no.	
Constituent	A	В
WE-NH : +		
Initial com	0	0
Processed corn	0.07	0.11
Ammoniacal nitrogen:		
Initial corn	0.04	0.06
Processed corn	0.32	0.39
Total nitrogen: ‡		
Initial corn	1.42	1.50
Processed corn	1.72	1.71
Reducing sugars: ‡§		
Initial corn	0.34	0.32
Processed corn	0.32	0.24
Nonreducing sugars:		
Initial corn	1.16	1.22
Processed corn	0.94	1.11
Soluble solids: ‡		
Initial corn	6.0	5.9
Processed corn	7.3	7.1
Linoleic acid, percent:**		
Initial com	48.6	59.1
Processed corn	43.5	53.9
Noneluted material++	6.8	6.0

^{*} All analyses reported as percent, dry basis, based on single determinations unless specified otherwise.

ammoniated, dried corn, increases occurred in WE-NH₃, NH₃-N, total nitrogen, and soluble solids contents (Table 3). The largest increase, 5 to 20 percent, occurred in the soluble solids content. Peplinski et al. (1975) reported almost a 50 percent increase in soluble solids content during a 14-month storage test of high-moisture corn treated with ammonia as a grain preservative, and a twofold increase in nitrogen content of the solubles. The authors proposed that soluble ammoniacal reaction products accounted for the increase in soluble solids content.

Increases in the WE-NH₃ content were small in the dried corn. The levels were sufficiently low for good acceptance by swine (Norred, 1977). Jensen et al. (1977) also had good acceptance when corn of similar WE-NH3 content from two shakedown runs was fed in a ground, mixed feed. Total nitrogen content increased by 0.2 to 0.3 percentage points (i.e., a 12 to 21 percent increase) while NH₃-N content increased about 0.3 percentage point. In feeding ruminants, this additional nitrogen might be the equivalent of an additional 1.2 to 1.8 percent protein. Small decreases of 0.1 to 0.2 percentage points occurred in both nonreducing sugar (reported as sucrose) and reducing sugar (reported as dextrose) contents. Ammoniation had no significant effect on the percentages of ash, crude fiber, or petroleum-ether extractable fat.

A small but statistically significant decrease occurred in linoleic acid content of the extracted fat, linoleic acid being a nutritionally essential fatty acid. Peplinski et al. (1975) observed a similar but greater change in fat composition and a large reduction in amount of petroleum ether-extractable fat during storage of the ammoniated high-moisture corn. More recently, Black et al. (1978)

TABLE 4, COLOR CHANGES IN CORN

Corn ammoniated	Color readings*		
ammoniated	L	+a	÷b
	Yellow corn-whole		
No	57.3	10.5	25.4
Yes	32.2	7.2	11.2
	Yello	w corn—gr	ound
No	80.3	0.6	24.6
Yes	70.7	1.2	19.4

^{*} Measurements made with Hunterlab color difference meter. L measures lightness (i.e., light reflectance) on the scale of 100 for perfect white, and 0 for black; +a measures degree of red coloration; and +b measures vellowness.

observed that air (oxygen) in sufficient quantity must be present for these reactions to occur.

A statistical analysis of data on amino acid composition of the corn before and after ammoniation indicated a 14 percent reduction in cystine and 11 percent reduction in lysine contents of the ammoniated corn. The analysis was based on samples from the four ammoniation runs, two hydrolysates per sample, and chemical analysis of two aliquots from each hydrolysate. Nitrogen recovered as amino acids decreased from 94 to 81 percent (overall averages) as a result of the ammoniation. Whether an actual reduction in amino acid content occurred or whether the analysis was affected by an artifact is currently unknown.

A small proportion of the ammoniated kernels had a light greyish coating which was easily removed by rubbing. When individual kernels from seven samples taken from lot ACA (run A) were surface sterilized and plated on malt extract for 5 days at 28 °C, no mold growth was det :ted. This finding is in agreement with the reported destruction of molds in corn by ammonia (Bothast et al., 1973). Furthermore, bacteria grew from only 19 percent of the kernels (average value) as compared with 100 percent that normally would be expected for the corn before ammoniation. The bacterium was not identified, but the presence of spore-forming rods indicates a *Bacillus* species (Bothast, 1976). Source of the greyish coating has not been determined, but it apparently did not arise from microbial activity.

Corn Physical Condition

The most obvious effect of ammoniation was the change in color of the corn from yellow to light brown. Color measurements on the whole grain showed a decrease in light reflectance and in intensity of both the yellow and red colors (Table 4). Examination of individual kernels revealed that the greatest color change took place in the pericarp or bran layers, moderate darkening occurred in the germ, and a tinge of darkening could be seen in the outer portions of the endosperm. The color change makes for ready identification of ammoniated corn.

Because the pericarp constitutes only about 6 percent of the kernel, measurements made on ground samples showed that a small decrease occurred in the yellow color whereas the red color increased slightly.

Corrosion

Interior galvanized iron surfaces in the ammoniation bin not coated with epoxy paint were severely attacked

[†]Water-extracted ammonia.

[‡]Average of duplicate determinations.

^{\$} Reported as dextrose.

^{||} Reported as sucrose.

^{**}As percent of fatty acids in hexaneextracted fat.

^{††}Percent of fat sample not eluted from the gas chromatographic column.

TABLE 5. ESTIMATED OPERATING COSTS FOR AMMONIATING CORN TO INACTIVATE AFLATOXIN

Item	\$/week*	Cents/original bushel†
Ammonia (33,075 lb at \$0.09/lb)	2,976.75	6.6‡
Electricity (25,000 kWh at \$0.05/kWh)	1,250.00	2.8
Fuel (propane) (4977 gal at \$0.38/gal)	1,891.26	4.2 §
Labor (300-man-hr at \$8.00/h)	2,400.00	5.3
Maintenance (5 percent/yr on \$345,000) Fixed charges (depreciation, taxes and	862.50	1.9
insurance), 13 percent/yr on \$345,000	2,242.50	5.0
Estimated operating costs, \$/wk Estimated operating costs,	\$11,623.01	
cents/original bushel		25.8

^{*}Based on operating 20 weeks per year.

by the ammonium hydroxide condensate. Portions of the roof panels in the ammoniation bin were completely denuded of galvanized coating after four runs were completed. However, the wall, floor, and plenum surfaces coated with the epoxy paint withstood the ammonia attack very well. Based upon our limited experience, the epoxy paint gives good protection.

Animal Feeding Experiments

Corn prepared in the current large-scale ammoniation experiments has been fed to swine and laying hens with highly satisfactory (Norred, 1977; Keyl and Norred, 1978; Keyl, 1978). As mentioned above, Jensen et al. (1977) also reported satisfactory acceptance and performance by swine fed ammoniated corn.

Preliminary Cost Estimate

The cost for decontaminating the corn should be considered relative to the cost and loss involved if the corn cannot be marketed or utilized but instead must be disposed of by burial or incineration. A preliminary cost estimate was prepared (late 1975 basis for equipment, September 1978 basis for labor, NH₃ and propane) for a hypothetical plant which would detoxify corn by operating 20 wk each year during warm weather. The plant would have an annual capacity of 31,720 m³ (900,000 bu). It presumably would operate as an adjunct to an existing grain elevator or feed mill. The general operating procedure would be as follows: (a) Clean the corn by a conventional method and assume a 3 percent

TABLE 6. ESTIMATED FIXED CAPITAL INVESTMENT FOR SYSTEM TO AMMONIATE CORN ON SCALE OF OPERATION USED IN TABLE 5

Equipment and installation costs	Cost, \$
Bins (12), installed, with accessories, 15,000 b	u
capacity each*	216,000
Three recycle blowers	63,000
Grain cleaner	6,000
Continuous dryer	30,000
Conveyors, instruments, elevators, etc.	6,000
Electrical installation	10,000
Contingencies	14,000
Estimated fixed capital investment	\$345,000

^{*}Each bin is installed on a concrete pad, insulated, interior surfaces coated with epoxy paint, and equipped with a vertical-screw, bin-stirring device and a grain-unloading auger. Three bins fitted with recycle piping, blower, etc, will be used to ammoniate the corn, and nine bins will hold the ammoniated corn until it is to be dried.

loss as screenings. (b) Temper the corn in two steps from 12-13 to 18 percent moisture, and blend the corn. Assume a 6-h holding period after the first addition of water and 12 h after the second. (If the corn already contains 18 to 20 percent moisture, this step can, of course, be omitted.) (c) Add 1.5 kg anhydrous NH₃ per 100 kg corn dry matter over a 6- to 12-h period. Recycle the gaseous NH₃-air mixture for 48 h in 530-m³ (15,000-bu) bins. Total NH₃ addition and recycling) is 13 days at 25 to 40 °C. Ammoniation is expected to lower the aflatoxin B₁ content from 750-1000 μ g/kg to less than 15 μ g/kg. (d) Dry the ammoniated corn to about 13 percent moisture and bring the WE-NH₃ content down to 0.25 percent, dry basis, or less.

When corn is ammoniated in batches of 382 t (530 m³) each, three batches per week, and 20 wk per yr (22,910 t annual capacity), the estimated operating costs are about 26 cents/bu of incoming corn (Table 5), or \$10.05/t. Estimated fixed capital investment for the plant is \$345,000 (Table 6).

Items included in the estimate are ammonia, utilities, labor, maintenance, and fixed charges. Not included are cost of corn, administrative expense, interest on investment, profit, and cost of the following: value of screenings and cost of their disposal, pollution control of dust and ammonia, recovery of ammonia from the dryer exhaust air if this should be necessary, and safety equipment. Ammonia, fuel, labor, and fixed charges are the largest cost items in the estimate. If, instead of propane gas, natural gas at \$5.30/100 m³ (\$1.50/1000 ft³) is used, the reduction in fuel costs would be a little over 2 cents/bu. Because of the caking problem, labor for removal of grain from the ammoniation bin is the single largest labor requirement in the process. The use of a vertical screw grain-stirring device presumably will prevent caking in the bin and thus reduce the labor requirements considerably, but procurement and installation of electrical devices and wiring that will be adequately protected from the ammonia may be a problem.

CONCLUSIONS

We successfully reduced the aflatoxin B_1 content of a 29-t batch of naturally contaminated corn from 750 to 7 μ g/kg by a recycle gas-phase treatment whereby we added 1.5 percent ammonia. Total aflatoxin (i.e., B_1 , B_2 , G_1 , and G_2) content of the ammoniated corn could not be determined because of the presence of interfering

[†]Original bushel is corn before screenings were removed.

[‡]One-cent change in ammonia price changes cost per bushel about 0.7 cent.

 $[\]S$ If natural gas at \$1.50/1000 cubic feet is used, fuel cost becomes about 1.5 cent/bu.

materials, but the residual level appears to be well below the current action guideline set by FDA, namely 20 ppb (i.e., $20 \mu g/kg$). Heated air drying as used for drying corn on the farm proved satisfactory for deodorizing the ammoniated corn.

Usage of the corn now appears limited to animal feeding. Feeding tests made by others, either completed or in progress, indicate good animal performance and acceptance by swine and laying hens of the processed corn and inactivation of the aflatoxin.

The estimated cost for decontaminating the corn, exclusive of some charges such as interest on investment, is 26 cents/bu for a plant processing 900,000 bu per year. Detoxification is one means of salvaging corn that cannot be marketed and is an alternative to disposal by burial or incineration.

Additional work is necessary on several processing problems and the solutions appear to be attainable.

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